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Use of Ribotyping To Distinguish *Bordetella bronchiseptica* Isolates

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A total of 113 *Bordetella bronchiseptica* strains, isolated from 11 different host species worldwide, were characterized by ribotyping with restriction enzyme *Pvu*II. Sixteen distinct ribotypes were identified, and each ribotype contained five to seven restriction fragments ranging in size from 1.8 to 5.6 kb. Approximately 88% of the swine isolates were identified as ribotype 3 strains. Isolates from dogs also displayed little variation; 74.1% were found to be ribotype 4 strains. Strains obtained from the remaining nine host species belonged to 15 different ribotypes. There was no association between geographic location and ribotype. The technique which we used may be useful for epidemiologic studies in which the transmission of *B. bronchiseptica*, both within and between species, is investigated.

Bordetella bronchiseptica is a common pathogen of the upper respiratory tract in a number of mammalian species. It causes atrophic rhinitis and pneumonia in swine, acute tracheobronchitis in dogs, and bronchopneumonia in a variety of laboratory animals, including rabbits, guinea pigs, rats, mice, cats, and nonhuman primates (8). On rare occasions, *B. bronchiseptica* is also associated with sinusitis, tracheobronchitis, pneumonia, and septicemia in humans (5, 10, 28).

Transmission of *B. bronchiseptica* is thought to occur primarily through aerosol droplets and direct contact between infected and noninfected animals (7). Cross-species transmission has been proposed as one mechanism of spread, but the frequency and importance of this process in the dissemination of disease are not known (4, 7). Since *B. bronchiseptica* is able to survive and even grow under nutrient-poor conditions, it has also been postulated that soil and water environments act as natural reservoirs for infection (15, 21, 22). However, there have been few epidemiologic studies which have addressed the mechanisms of transmission of this organism.

A major obstacle to understanding the natural transmission patterns of *B. bronchiseptica* is the lack of a simple and reliable typing system for classification of various isolates. Some investigators have attempted to utilize phenotypic properties of *B. bronchiseptica* to distinguish between isolates from different hosts. Pederson (19) conducted a serotyping study in which it was demonstrated that there is heterogeneity among pig, cat, rabbit, mouse, and rat isolates. However, the usefulness of serotype-specific antisera for establishing host specificity remains questionable, since only one or a few isolates of each species were tested. Although several other workers have also demonstrated that there is an association between particular phenotypes and host species (3, 6, 17, 23), a serious limitation of such approaches for classification of strains is that phenotypic traits of bacteria can vary under different growth conditions. This is especially true for *Bordetella* species, which undergo extensive alterations in surface proteins and biological characteristics during culture in vitro (1, 20).

Multilocus enzyme electrophoresis (MLEE) has also been used to determine the degree of variation in *B. bronchiseptica* strains (17). Like serotyping, this technique depends upon phenotypic expression of genetic loci. However, expression of the metabolic enzymes upon which classification is based is thought to be more stable than expression of surface antigens (24). In a separate study, evidence supporting the stability of *Bordetella pertussis* electrophoretic types (ETs) in vitro was obtained under conditions that induced a variety of other phenotypic changes (18). It has been assumed that *B. bronchiseptica* ETs are similarly invariant, although this has not been tested experimentally. MLEE has the additional disadvantage of being technically cumbersome, since several different buffer systems must be tested for optimal results and numerous staining solutions are required (24). Depending on the degree of polymorphism, several hundred gels may be necessary for an analysis of 100 isolates (24).

Methods that rely on analysis of stable genetic elements are likely to be more reproducible for classification of bacterial strains than expression-based techniques. However, the utility of such techniques for classification of bordetellas is questionable, since a comparison of chromosomal DNA sequences based on reassociation reactions indicated that *B. pertussis*, *ordetella parapertussis*, and *B. bronchiseptica* are closely related and can be considered members of a single genomic group (13). The results of the same study also suggested that the genetic variance among *B. bronchiseptica* isolates from three different host species was minimal, although only a few strains were analyzed. In addition, this technique may not be sufficiently discriminatory to distinguish between closely related populations, since the level of experimental error can be as high as 10% (24, 25). Conversely, the results of a study in which pulsed-field gel electrophoresis was used revealed three distinct DNA types among the eight *B. bronchiseptica* isolates tested (12). Since the origin of these strains was not given, it is unclear whether there was any correlation between DNA type and host species. The fragmentary and conflicting data currently available do not clearly establish whether the level of sequence variability among *B. bronchiseptica* isolates is sufficient to permit placing strains into different groups.

Analysis of restriction fragment length polymorphisms of rRNA genes, or ribotyping, is a widely used technique for differentiating bacterial strains (9, 27). The goal of our study was to determine whether this technique can be used to differentiate isolates of *B. bronchiseptica* obtained from a variety of host species.

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TABLE 1. *B. bronchiseptica* strains used

Strain	Host	Country of origin	Ribotype	ET[a]
MBORD545	Pig	The Netherlands	3	1a
MBORD553	Pig	United Kingdom	1	1a
MBORD603	Pig	Canada	3	1
MBORD605	Pig	Canada	3	1
MBORD606	Pig	Canada	3	1
MBORD676	Pig	Australia	2	3
MBORD677	Pig	Australia	3	3
MBORD688	Pig	United States	3	8
MBORD790	Pig	The Netherlands	3	1
MBORD791	Pig	The Netherlands	3	1
MBORD792	Pig	The Netherlands	3	1
MBORD793	Pig	The Netherlands	3	1
MBORD795	Pig	The Netherlands	3	1
MBORD796	Pig	The Netherlands	3	1
MBORD797	Pig	The Netherlands	3	1
MBORD798	Pig	The Netherlands	3	1
MBORD800	Pig	The Netherlands	3	1
MBORD801	Pig	The Netherlands	3	1
MBORD802	Pig	The Netherlands	3	1
MBORD803	Pig	The Netherlands	3	1
MBORD804	Pig	The Netherlands	3	1
MBORD805	Pig	The Netherlands	3	1
MBORD846	Pig	Switzerland	2	1
MBORD847	Pig	Switzerland	2	1
MBORD849	Pig	The Netherlands	3	1
MBORD850	Pig	The Netherlands	3	1
MBORD853	Pig	Ireland	3	1
MBORD976	Pig	The Netherlands	3	1
MBORD979	Pig	The Netherlands	3	1
MBORD980	Pig	The Netherlands	3	1
B58	Pig	Hungary	3	ND[b]
B65	Pig	Hungary	3	ND
5203	Pig	Hungary	3	ND
MBORD590	Dog	United States	4	6
MBORD591	Dog	United States	13	14
MBORD592	Dog	United States	4	6
MBORD594	Dog	United States	4	6
MBORD595	Dog	United States	5	4
MBORD596	Dog	United States	4	6
MBORD599	Dog	United States	4	6
MBORD600	Dog	United States	4	6
MBORD601	Dog	United States	4	6
MBORD602	Dog	United States	4	6
MBORD685	Dog	United States	3	1
MBORD686	Dog	United States	3	1
MBORD732	Dog	Denmark	4	8
MBORD748	Dog	Denmark	4	8
MBORD749	Dog	Denmark	4	16
MBORD750	Dog	Denmark	4	16
MBORD783	Dog	The Netherlands	4	16
MBORD785	Dog	The Netherlands	3	1
MBORD786	Dog	The Netherlands	4	8
MBORD787	Dog	The Netherlands	4	16
MBORD788	Dog	The Netherlands	13	8
MBORD827	Dog	Switzerland	4	6
MBORD839	Dog	Switzerland	4	8
MBORD843	Dog	Switzerland	4	6
MBORD965	Dog	The Netherlands	4	1
MBORD966	Dog	The Netherlands	3	1
MBORD967	Dog	The Netherlands	4	8
MBORD671	Rabbit	United States	9	ND
MBORD704	Rabbit	United States	2	1
MBORD705	Rabbit	United States	5	ND
MBORD730	Rabbit	Denmark	9	16
MBORD784	Rabbit	The Netherlands	3	1
MBORD823	Rabbit	Switzerland	3	1
MBORD828	Rabbit	Switzerland	3	1
MBORD831	Rabbit	Switzerland	9	16
MBORD833	Rabbit	Switzerland	12	1
MBORD834	Rabbit	Switzerland	12	1
MBORD835	Rabbit	Switzerland	9	16
MBORD836	Rabbit	Switzerland	3	1
MBORD837	Rabbit	Switzerland	9	16
MBORD838	Rabbit	Switzerland	12	1
MBORD971	Rabbit	The Netherlands	9	16
MBORD972	Rabbit	The Netherlands	3	1
MBORD981	Rabbit	The Netherlands	3	1
MBORD629	Cat	United States	4	16
MBORD630	Cat	United States	4	16
MBORD631	Cat	United States	4	16
MBORD635	Cat	United States	2	1
MBORD723	Cat	Denmark	4	16
MBORD733	Cat	Denmark	4	16
MBORD745	Cat	Denmark	3	1
MBORD782	Cat	The Netherlands	13	ND
MBORD968	Cat	The Netherlands	4	16
MBORD970	Cat	The Netherlands	13	16
MBORD627	Guinea pig	United States	9	16
MBORD665	Guinea pig	United States	11	16
MBORD666	Guinea pig	United States	12	16
MBORD668	Guinea pig	United States	12	16
MBORD669	Guinea pig	United States	3	16
MBORD670	Guinea pig	United States	2	3
MBORD673	Guinea pig	Germany	3	3
MBORD674	Guinea pig	Germany	12	1
MBORD762	Guinea pig	Ireland	10	1
MBORD854	Guinea pig	Switzerland	10	1
MBORD624	Horse	United States	7	16
MBORD628	Horse	United States	4	16
MBORD632	Horse	United States	9	16
MBORD633	Horse	United States	4	16
MBORD731	Horse	Denmark	8	ND
MBORD982	Horse	The Netherlands	8	ND
MBORD983	Horse	The Netherlands	4	ND
MBORD681	Koala	Australia	6	1
MBORD698	Koala	Australia	6	1
MBORD700	Koala	Australia	6	1
MBORD675	Human	Germany	14	14
St. Louis	Human	United States	15	ND
MBORD707	Turkey	United States	16	1
MBORD901	Turkey	Germany	15	ND
MBORD625	Rat	United States	2	16
MBORD626	Leopard	United States	6	16

[a] ET as described by Musser et al. (17).
[b] ND, not determined.

MATERIALS AND METHODS

Strains. A total of 113 *B. bronchiseptica* isolates were examined. Strain St. Louis was provided by Tom Milligan, St. Louis University Hospital, St. Louis, Mo., and was identified on the basis of Gram staining, colony morphology, and the results of standard biochemical tests. Strains B58, B65, and 5203 (14) were obtained from Tibor Magyar, Veterinary Medical Research Institute of the Hungarian Academy of Sciences, Budapest, Hungary. All of the remaining strains were originally characterized by workers in the laboratory of James Musser (17) and were generously provided by David Dyer, University of Oklahoma, Oklahoma City. The strains were obtained from a variety of host species and from geographically diverse locations (Table 1). They included 33 swine isolates, 27 dog isolates, 17 rabbit isolates, 10 cat isolates, 10 guinea pig isolates,

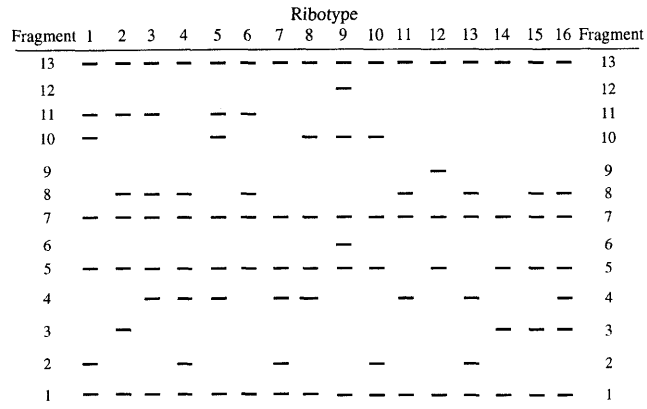

FIG. 1. Ribotype patterns observed following digestion of *B. bronchiseptica* chromosomal DNAs with *Pvu*II. A total of 13 unique fragments were observed, and these fragments ranged in size from 1.8 kb (fragment 1) to 5.6 kb (fragment 10).

7 horse isolates, 3 koala isolates, 2 turkey isolates, 2 human isolates, 1 leopard isolate, and 1 rat isolate. All of the strains were cultivated for 36 to 48 h at 37°C on Bordet-Gengou medium supplemented with 15% defibrinated sheep blood.

Purification of chromosomal DNA. A single loopful of bacterial growth was resuspended in 0.5 ml of phosphate-buffered saline and washed once. Genomic DNA was purified by using a commercially available kit as recommended by the manufacturer (QIAGEN, Inc., Chatsworth, Calif.). Final DNA precipitates were dissolved by overnight incubation at 55°C in 10 mM Tris–1 mM EDTA (pH 8.0). The concentrations and purities of DNA preparations were assessed spectrophotometrically.

Restriction endonuclease digestion and gel electrophoresis. Several restriction enzymes were used in preliminary experiments to identify one or more enzymes that gave optimal results. A 3-μg portion of DNA was digested with 10 U of either *Eco*RI, *Sal*I, *Pst*I, *Hin*fI, *Alu*I, or *Pvu*II at 37°C overnight. After incubation, loading dye (0.25% bromphenol blue, 30% glycerol) was added, and samples were electrophoresed in 0.6% agarose gels containing 0.5 μg of ethidium bromide per ml in Tris-borate buffer (89 mM Tris, 89 mM boric acid, 2 mM disodium EDTA). Following electrophoresis, DNA was visualized by UV illumination and photographed with type 57 Polaroid film to confirm that the high-molecular-weight nucleic acid was completely digested and to establish that visually equivalent amounts of DNA were present in the lanes. A 1- to 12-kb DNA ladder (Boehringer Mannheim) was included in an adjacent lane on each gel, and the migration distances of the fragments were used to prepare a standard curve. The molecular masses of *B. bronchiseptica* fragments were determined by plotting the migration of the fragments on standard curves.

Southern blots. After electrophoresis, DNA was partially depurinated by incubating it in 250 mM HCl for 10 min and then was denatured in 0.5 N NaOH–1.5 M NaCl. The gel was neutralized in 1.0 M Tris (pH 8.0)–1.5 M NaCl. Restriction fragments were transferred to charged nylon membranes by overnight capillary transfer by using 10× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate, pH 7.0), as described previously (26). After this transfer, the DNA was fixed to the membranes by UV cross-linking with a UV Stratalinker (Stratagene, La Jolla, Calif.) as recommended by the manufacturer.

Ribotyping. Plasmid pRRNB (generously provided by Kenneth Pidcock, Wilkes University, Wilkes-Barre, Pa.) was digoxigenin labeled by the random priming method (2) and was hybridized to membrane-bound chromosomal DNA digests. This plasmid contains a 5.4-kb fragment of the *Escherichia coli* rRNA operon *rrnB* (16). Prehybridization and hybridization were carried out at 42°C, as described previously (2), in a maleic acid-based buffer. The hybridization solution contained 10 ng of digoxigenin-labeled probe per ml in 5× SSC–50% formamide–0.02% sodium dodecyl sulfate (SDS)–0.1% *N*-lauroylsarcosine–2% blocking reagent–20 mM sodium maleate. Following removal from the hybridization solution, the membranes were washed twice for 5 min at room temperature in 2× SSC containing 0.1% SDS and twice for 15 min at 65°C in 0.5× SSC containing 0.1% SDS. Bound probe was detected by using an anti-digoxigenin–alkaline phosphatase antibody conjugate and LumiPhos as described previously (2). Membranes were sealed in plastic bags prior to exposure to Kodak XAR5 film. Following detection of bound pRRNB, the membranes were stripped of the probe by incubating them at 37°C for 30 min in 0.2 N NaOH–0.1% SDS. The membranes were subsequently hybridized with digoxigenin-labeled pUC19, the vector containing the *rrnB* fragment, to verify that positive hybridization signals were due to the fragment and not to vector sequences.

Data analysis. Ribotypes were designated by Arabic numerals, which were assigned as the ribotypes were encountered. Strains that exhibited single band differences were assigned to different ribotypes. The discriminatory power of ribotyping for isolates derived from various groups of host species was defined by calculating discrimination indices as described by Hunter and Gaston (11).

RESULTS AND DISCUSSION

Ribotype identification. The initial experiments were performed with a subset of *B. bronchiseptica* strains to identify a restriction enzyme that provided multiple, easily defined band patterns. DNAs from 10 strains representing the pig, dog, and rabbit isolates were digested with *Eco*RI, *Sal*I, *Pst*I, *Hin*fI, *Alu*I, or *Pvu*II, and this was followed by Southern blotting and hybridization with pRRNB. *Pvu*II was found to provide the largest number of easily separated fragments and was used for the remainder of the study.

Sixteen distinct band patterns resulting from different combinations of 13 unique restriction fragments were identified when the 113 strains listed in Table 1 were examined. A representation of each ribotype pattern is shown in Fig. 1. Each ribotype consisted of five to seven fragments that ranged in size from 1.8 kb (fragment 1) to 5.6 kb (fragment 13). Fragments 13, 7, and 1 were conserved in all strains. Examples of 7 of the 16 band patterns are shown in Fig. 2.

The band patterns representing ribotypes 3 and 4 were the most commonly observed band patterns (37.2 and 25.7% of all isolates, respectively). The remainder of the strains were scattered among 14 other ribotypes, with no more than 7.1% of the strains in any single group.

Approximately one-half of the strains used in this study were subjected to repeat analysis to assess the reproducibility of ribotyping. The pattern obtained was always identical to the original pattern for each strain (data not shown). In addition, at least one strain representing each ribotype has been used repeatedly as a type standard in our laboratory during the past 1 year, with invariant results.

Distribution of host species within ribotypes. Table 2 shows the distribution of host species within ribotypes. A total of 87.9% of the swine strains were ribotype 3 strains; the remaining swine isolates were ribotype 1 or 2 strains. Conversely, 69.0% of the strains identified as ribotype 3 isolates were obtained from pigs. Ribotype 3 also included a minority of the strains from dogs, cats, and guinea pigs. In addition, ribotype 3 was one of the two predominant groups containing rabbit isolates.

Strains isolated from dogs were also highly associated with a single ribotype; 74.1% of the isolates were ribotype 4 strains, while the remaining isolates tested were ribotype 3, 5, or 13 strains. Dog strains accounted for 69.0% of the ribotype 4 isolates; the remainder of the strains in this group were originally isolated from either cats or horses.

All of the strains isolated from koalas were ribotype 6 strains. Since only three isolates from this host were available

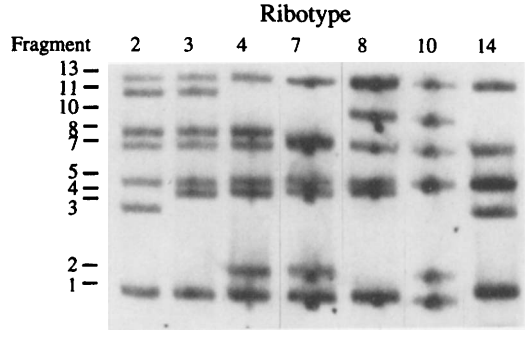

FIG. 2. Lumigraph showing the results of one ribotyping experiment in which seven different ribotypes were identified. *B. bronchiseptica* chromosomal DNAs were digested with *Pvu*II.

TABLE 2. Distribution of host species within ribotypes

Ribotype	No. of strains from the following hosts											Total no. of strains
	Pig	Dog	Rabbit	Cat	Guinea pig	Horse	Koala	Human	Turkey	Rat	Leopard	
1	1											1
2	3		1	1	1					1		7
3	29	4	6	1	2							42
4		20		6		3						29
5		1	1									2
6							3				1	4
7						1						1
8						2						2
9			6		1	1						8
10					2							2
11					1							1
12			3		3							6
13		2		2								4
14								1				1
15								1	1			2
16									1			1
Total	33	27	17	10	10	7	3	2	2	1	1	

for testing, there are not enough data to establish that there is a strong association between koala isolates and ribotype 6.

There was no obvious correlation between any other host species and a single ribotype pattern.

Geographic distribution of ribotypes. It is evident from the information presented in Table 1 that a number of different ribotypes occur in most of the countries represented in this study. Thus, there is no obvious association between ribotype and geographic location. Although only a single ribotype was found among the isolates from Hungary and Canada, only a few isolates from these countries were available for testing.

Comparison of ribotyping with MLEE. MLEE, a technique that detects mobility variants of metabolic enzymes, is the only method that has been used previously to determine the degree of variation in a large number of *B. bronchiseptica* strains (17). In the study of Musser et al. (17), 303 isolates from 11 different host species were classified into 21 different groups or ETs. Since this technique could prove to be useful for epidemiologic purposes, we used the data of Musser et al. (17) to compare the discriminatory power of MLEE with that of ribotyping.

When ribotyping data derived from the 113 strains used in this study were analyzed, a discrimination index of 0.786 was obtained (Table 3). This value indicates that two randomly isolated strains would fall into different ribotypes 78.6% of the time. A method is considered sufficiently discriminatory for typing purposes if a discrimination index of 0.900 or greater is obtained (11). The discrimination index for MLEE, based on the data provided in the study of Musser et al. (17), is 0.567. Although neither the ribotyping method nor the MLEE method yielded a level of confidence suitable for epidemiologic studies, ribotyping is more discriminatory. It appeared that the strong association of pig isolates and dog isolates with ribotypes 3 and 4, respectively (Table 2), significantly impaired the ability of the ribotyping method to differentiate random isolates. Similarly, MLEE revealed low levels of interspecies clonal diversity with pig and dog isolates (17). The discrimination indices for strains isolated from only these two species were also very low (Table 3). Therefore, we calculated discrimination indices for ribotyping and MLEE based on the results obtained with isolates from all of the host species except pigs and dogs. For ribotyping, the index was high enough (0.905) that this method can be used with confidence to monitor transmission patterns of *B. bronchiseptica* strains in hosts other than pigs and dogs. Even when isolates from pigs and dogs were omitted from the analysis, the discriminatory ability of MLEE was not sufficient to propose that this method could be used as an epidemiologic tool (Table 3).

The superior ability of ribotyping for distinguishing *B. bronchiseptica* isolates is further illustrated in Table 4. The ETs for 102 of the 113 strains used for ribotyping were obtained from the report of Musser et al. (17), and their distribution within ribotypes was determined. The strains in the two most common ETs, ETs 1 and 16, could be further differentiated into seven or eight additional groups when ribotyping was employed. In con-

TABLE 3. Discrimination indices for ribotyping and MLEE of *B. bronchiseptica* isolates[a]

Typing method	No. of host species	No. of types	No. of isolates in different types	Discrimination index
Ribotyping	11[b]	16	1, 7, 42, 29, 2, 4, 1, 2, 8, 2, 1, 6, 4, 1, 2, 1	0.786
	2[c]	6	1, 3, 33, 20, 1, 2	0.592
	9[d]	15	4, 9, 9, 1, 4, 1, 2, 8, 2, 1, 6, 2, 1, 2, 1	0.905
MLEE	11[b]	21	193, 1, 4, 7, 1, 32, 1, 1, 1, 1, 1, 1, 7, 7, 1, 38, 1, 1, 1, 1, 2	0.567
	2[c]	15	169, 3, 6, 1, 30, 1, 1, 1, 4, 3, 1, 1, 1, 1, 2	0.418
	9[d]	13	24, 1, 1, 1, 2, 1, 1, 1, 3, 4, 1, 37, 1	0.683

[a] Ribotypes were determined in this study. MLEE indices were calculated by using the data of Musser et al. (17).
[b] Strains from all host species were tested.
[c] Only pig and dog isolates were tested.
[d] Strains from all host species except pigs and dogs were tested.

TABLE 4. Distribution of MLEE ETs within ribotypes[a]

Ribotype	No. of strains with the following MLEE ETs						
	1	3	4	6	8	14	16
1	1						
2	4	2					1
3	35	2			1		1
4	1			10	5		12
5			1				
6	3						1
7							1
8							
9							7
10	2						
11							1
12	4						2
13					1	1	1
14						1	
15							
16	1						
Total	51	4	1	10	7	2	27

[a] The MLEE ETs for 102 of the 113 strains used for ribotyping were obtained from the report of Musser et al. (17).

trast, the two most prevalent ribotypes, ribotypes 3 and 4, were separated into only three additional groups when MLEE results were considered.

The data in Table 4 also illustrate that a combination of ribotyping and MLEE can be used to define 26 distinct groups for the 102 isolates included in both studies, compared with only 7 MLEE groups and 14 ribotypes. Therefore, the use of these two methods together is likely to provide superior discriminatory power compared with ribotyping alone. The calculated discrimination index for MLEE combined with ribotyping was 0.853, indicating that the combination of typing methods is superior to ribotyping alone. However, this combination of techniques cannot be recommended for routine typing of *B. bronchiseptica* isolates due to the labor-intensive nature of MLEE and to the fact that the discrimination index still falls short of the value recommended as acceptable for a typing method (11).

Ribotyping has been used by many investigators for determining the molecular epidemiology of genetically diverse organisms (9, 27). Our data demonstrate that there is sufficient diversity within *B. bronchiseptica* to allow grouping of isolates into distinct types. Based on the distribution of ribotypes within host species, we were unable to demonstrate sufficient diversity within isolates from pigs and dogs to permit documentation of transmission patterns in homologous species. The results of MLEE also indicated that strains from pigs and dogs are highly clonal (17). This lack of genetic divergence may be useful in some situations. If an isolate of unknown origin is identified as a ribotype 3 strain, there is a strong probability that it originated in swine. Isolates with a ribotype 4 pattern are likely to have arisen in dogs. The small number of isolates from other hosts that also produced these ribotype patterns may be the result of cross-species transmission. However, any conclusions concerning the origin of isolates reached on the basis of identification as ribotype 3 or 4 strains must be considered tentative.

Our data also indicate that ribotyping may be an effective tool for molecular epidemiology in hosts other than pigs and dogs. While ribotyping requires the capability to perform agarose gel electrophoresis and Southern blotting, it is not a technically difficult procedure. Utilization of a digoxigenin-labeled probe, rather than a probe labeled radioactively, facilitates the use of this method since digoxigenin-labeled probes can be reused for 1 year or more and no special safety precautions or disposal procedures are required.

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